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(54) Title: HYPERICIN AND *HYPERICUM* EXTRACT: SPECIFIC T–TYPE CALCIUM CHANNEL BLOCKER, AND THEIR USE AS T–TYPE CALCIUM CHANNEL TARGETED THERAPEUTICS

(57) Abstract

Hypericin has been shown to specifically inhibit T–type calcium channel activity. *Hypericum* extract containing hypericin also inhibits T–type calcium channel activity. Moreover, other chemicals in *Hypericum* extract showed a synergistic effect to hypericin. In view of this, hypericin or hypericin–containing *Hypericum* extract can be used as T–channel blockers. *Hypericum* extract, extract of other species of the *Hypericum* genus, extract of other plants containing hypericin, hypericin derivatives, hypericin analogs, such as pseudohypericin, and other *Hypericum* extract constituents can be used as therapeutics targeted at T–type calcium channels for treatment of diseases associated with T–channel abnormality. Methods for administering hypericin and *Hypericum* extract are disclosed.

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Hypericin and *Hypericum* extract: Specific T-type calcium channel blocker, and their use as T-type calcium channel targeted therapeutics

Field of the Invention

This invention relates to *Hypericum perforatum*, extracts of *Hypericum perforatum,* compounds found in *Hypericum perforatum*, e.g. hypericin, and the derivatives and analogs of hypericin. One aspect of the present invention is the discovery that *Hypericum perforatum* (referred to as *Hypericum* herein after unless otherwise indicated), *Hypericum* extracts, certain compounds in *Hypericum*, including hypericin, pseudohypericin, hyperforin, ashyperforin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, hypericin derivatives and hypericin analogs can be used as therapeutics targeted at T-type calcium channels in various biological systems, such as cardiovascular system, central nervous system and endocrine system, to treat diseases treatable with T-type calcium blocking agents. The diseases treatable with T-type calcium blocking agents include depression, chronic heart failure, congestive heart failure, ischaemc condition, arrhythmia, angina pectoris, hypertension, hypo- and hyperinsulinemia, diabete mellitus, hyperaldosteronemia, epilepsy, migraine headache, brain aging or neurodegenerative related diseases, such as Alzheimer's disease, and preterm labor.

Background of the Invention.

Hypericin is one of the chemical constituents from a perennial herbaceous plant, *Hypericum perforatum* or St. John's Wort. *Hypericum* is known to have medicinal properties since ancient times and it is widely used in phytotheraphy. *Hypericum* has been widely researched for its antidepressant and anti-viral properties. In addition to these properties, *Hypericum* has historically been used for a variety of neurological conditions, including anxiety, insomnia due to restlessness, irritability, neuralgia, trigeminal neuralgia, neuroses, migraine headaches, fibrositis, dyspepsia, and sciatica. *Hypericum* contains several compounds of biological interest, including naphthodianthrones, e.g. hypericin and

pseudohypericin, phloroglucinols, e.g. hyperforin and ashyperforin, and a broad spectrum of flavonoids which are considered to be primarily responsible for Hypericum's activity. However, the lack of a clearly definable pharmacologic mechanism of *Hypericum* and its chemical components cause the failure of identifying the constituents most responsible for *Hypericum*'s activity.

Clinical studies demonstrated that *Hypericum* is effective in treating mild depression. Animal studies also showed that *Hypericum* extract relieved depressant symptoms. It was reported that *Hypericum* extract resulted in a down-regulation of adrenergic receptors in the rat frontal cortex after subchronic treatment. Some reported that hypericin inhibited monoamine oxidase (MAO) activity in vitro, but others have failed to confirm this effect. Other proposed mechanisms involve effects on serotonin. At very high doses, *Hypericum* extract inhibited seretonin re-uptake although it is not known which chemical in the extract is responsible. Studies have shown that both hypericin and pseudohypericin inhibited a variety of virus. Hypericin has been reported to inhibit the growth of glioma cell lines in vitro and to be a potent inducer of glioma cell death due to inhibition of protein kinase C(PKC). Receptor tyrosine kinase activity of epidermal growth factor has also been reported to be inhibited by hypericin. These later effects have been linked to both the antiviral and antineoplastic activity.

It is known (F.R. Buhler, *J. Hypertension* supplement 15(5):s3-7, 1997; B. Cremers et al., *J. Cardiovascular Pharmacology*, vol. 29(5), pp. 692-6, 1997) that T-type channels are involved in pacemaker activity, low-threshold calcium spikes, neuronal oscillations and resonance, and rebound burst firing. It was reported that Mibefradil, a selective T-channel blocker, induces peripheral and coranary vasodilation. There is no reflex sympathetic activation and no negative inotropic effect. It increases coronary blood flow without increasing oxygen consumption and causes a slight slowing of the heart rate, thereby inducing diastolic relaxation. The

latter improves subendocardial and small artery perfusion. Ventricular ectopic activity is reduced with mibefradil. The renin-angiotensin-aldosterone system and endothelin effects are blunted by T-channel inhibition. It is believed that mibefradil could lead to a greater therapeutic index and greater safety over conventional non-selective or L-type calcium channel blockers in the treatment of cardiovascular diseases. Mibefradil has been used to treat hypertension and angina clinically. It was reported that Zonisamide, a antiepileptic drug reduces T-type calcium current (M. Kito et al., *Seizure, vol.* 5(2), pp. 115-9, 1996). T-type calcium channels also facilitate insulin secretion by enhancing the general excitability of these cells. Therefore, T-type calcium channels may be therapeutic targets in hypo- and hyperinsulinemia (A. Bhattacharjee et al., *Endocrinology*, vol. 138(9), pp. 3735-40, 1997). A direct link between T-type calcium channel activity and steroidogenesis has been suggested (M.F. Rossier et al., 1996).

Summary of the Invention

Consequently, T-type calcium channel blockers, such as *Hypericum, Hypericum* extracts, *Hypericum* constituents, including hypericin, pseudohypericin, hyperforin, ashyperforin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, hypericin derivatives and hypericin analogs of the present invention are effective in treating disorders characterized by an insufficiency of a steroid hormone.

Specific T-Channel inhibitory effect of hypericin and *Hypericum* extract containing 0.3% of hypericin have been found by the present inventors. The present inventors have unexpectedly found that hypericin and *Hypericum* extracts act as specific T-calcium channel blockers. In view of this, *Hypericum*, *Hypericum* extracts, extracts of species of the *Hypericum* genus other than *Hypericum perforatum*, extracts of other plants containing hypericin, constituents of *Hypericum*, including hypericin, hypericin derivatives and hypericin analogs according to the present

invention are expected to be useful for treating T-calcium channel targeted diseases such as arrthymia, coronary diseases, angina, hypertension, migraine, diabetes and preterm labor, etc.

Within the scope of the present invention are processes of using *Hypericum; Hypericum* extracts; *Hypericum* constituents, including hypericin, pseudohypericin, hyperforin, ashyperforin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin; hypericin derivatives; or hypericin analogs to treat health disorders related to T-type calcium channels or treatable with T-calcium channel blockers in animals, including humans. The health disorders related to T-type calcium channels or treatable with T-type calcium blocking agents include depression, chronic heart failure, congestive heart failure, ischaemc condition, arrhythmia, angina pectoris, hypertension, hypo- and hyperinsulinemia, diabete mellitus, hyperaldosteronemia, epilepsy, migraine headache, brain aging or neurodegenerative related diseases, such as Alzheimer's disease, and preterm labor.

Brief Description of Drawings

Figure 1 shows the effect of hypericin on L-type calcium current activity in cultured N1E-115 cells. At doses of 0.1, 1 and 10 uM, hypericin causes a slight but not significant increase in L-type calcium currents.

Figure 2. Shows the effect of nifedipine on L-type calcium current activity in cultured N1E-115 cells. The cells were added into nifedipine after not responding to hypericin (10uM). 1 uM of nifedipine significantly inhibited the currents, indicating the existence of L-type calcium channels.

Figure 3 shows the effect of hypericin on T-type calcium current in N1E-115 cells. Hypericin causes a dose-dependent inhibitory effect on T-type calcium current from 0.1 to 10 uM.

Figure 4 shows the effect of solvent control for hypericin on T-type calcium channel activity. Since hypericin is dissolved in a solvent of ethanol (50%)+DMSO (50%). This solvent is tested for its effect on T-type current.

In the amount equivalent to dissolving 0.1 to 10 uM of hypericin, this control solvent does not affect T-type calcium currents.

Figure 5 shows the effect of *Hypericum* extract on T-type calcium current. *Hypericum* extract contains about 0.3% of hypericin and is dissolved in external solution. At 50ug/ml, *Hypericum* extract significantly inhibited T-type calcium channel currents.

Figure 6 shows the effect of hypericin on L-type calcium current in vascular smooth muscle cells.

Figure 7 shows the effect of hypericin on L-type calcium current in ventricular cells from baby rats.

Detailed Description of the Invention

The present inventors have found that a commercially available standardized *Hypericum* extract containing the primary compounds, such as hypericin, pseudohypericin, flavonol glycosides, and phloroglucinols, etc, and pure hypericin inhibited T-type calcium channel activity in cultured neuroblastoma cells.

Hypericum extracts can be obtained either through commercially available sources or extracted from original whole fresh or dried *Hypericum* plant containing not less than 0.04% naphthodianthrones of the hypericin group calculated as hypericin. *Hypericum* extracts can be easily prepared by organic solvent extraction or supercritical fluid extraction by carbon dioxide (E. Bombardelli and P. Morazzoni, *Fitoterapia*, vol. 66, pp. 43-68, 1995; S.S. Chatterjee et al, *Pharmacopsychiat.*, vol. 31 (Suppl.), pp. 7-15, 1998; W. Dimpfel et al, *Pharmacopsychiat.*, vol. 31 (Suppl.), pp. 30-35, 1998). As an example, 1 kg of finely ground dried *Hypericum perforatum* was stirred with 8 l of 80% ethanol under nitrogen at 55°C for 1 hour. The mixture was centrifuged under nitrogen and the supernatant was collected. Ascorbic acid (0.1%) was added and the extract was dried under reduced pressure to give a *Hypericum* extract. It is noted that other organic solvents, e.g. alkyl alcohols other than ethanol, acetone, methyl n-butyl ketone, n-hexane, DMSO and toluene, can be used to extract

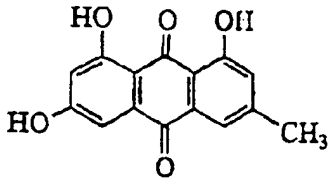
Hypericum perforatum to make *Hypericum* extracts. The plant, *Hypericum perforatum,* can be obtained worldwide such as England, China, Holland, French, German, Italy, Russia, Spain and Sweden.

Another embodiment of the present invention includes extracts from species of the *Hypericum* genus other than St. John's Wort or *Hypericum perforatum*, and extracts from other plants containing hypericin, as well as methods of using these extracts in treating health disorders related to T-type calcium channels or treatable with T-calcium channel blockers. Also within the scope of the present invention are methods of using species of the *Hypericum* genus, other than St. John's Wort (*Hypericum perforatum)*, or other plants containing hypericin to treat health disorders related to T-type calcium channels or treatable with T-calcium channel blockers.

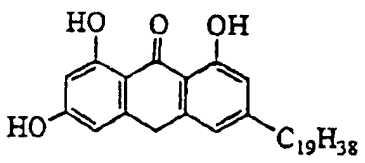
Hypericum extracts usually contain hypericin and various amounts of other chemicals, including pseudohypericin; a broad range of flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin; phloroglucinols, such as hyperforin and ashyperforin; the essential oil; and xanthones. In the present invention, *Hypericum* constituents include hypericin, pseudohypericin, flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, phloroglucinols, such as hyperforin and ashyperforin, the essential oil from *Hypericum perforatum*, and xanthones. Other embodiments of the present invention are *Hypericum* extract constituents, which include hypericin, pseudohypericin, flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, phloroglucinols, such as hyperforin and ashyperforin, the essential oil from *Hypericum perforatum*, and xanthones. Also within the scope of the present invention are methods of using *Hypericum* constituents or *Hypericum* extract constituents in treating health disorders related to T-type calcium channels or treatable with T-calcium channel blockers.

A further embodiment of the present invention includes hypericin derivatives and hypericin analogs. Chemicals other than hypericin in *Hypercum* extract may potentiate the biological effect of hypericin as proved by their synergistic effects of inhibiting T-type calcium channel activity. Also within the scope of the present invention are methods of using hypericin derivatives or hypericin analogs in treating health disorders related to T-type calcium channels or treatable with T-calcium channel blockers.

The term "hypericin analog" refers to compounds having a chemical structure similar to hypericin and having T-type calcium channel blocking activities like hypericin, but "hypericin analog" excludes Mibefradil. Examples of hypericin analogs include emodin and a compound of formula I shown below.

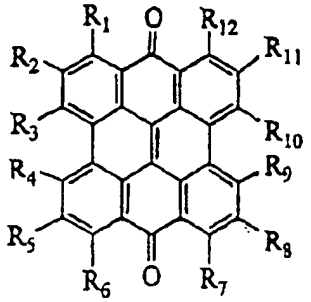


Emodin



I

In the present application, hypericin derivatives are compounds modified from hypericin. Pseudohypericin can be considered as a hypericin derivative. Hyperin and hypericin derivatives of the present invention include compounds of formula II shown below.



II

wherein

R_1 is H, OH, OR or OCOR;

R_2 is H, R, F, Cl, Br, I or SO_3H;

R_3 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_4 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_5 is H, R, F, Cl, Br, I or SO_3H;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H, R, F, Cl, Br, I or SO_3H;

R_9 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{10} is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{11} is H, R, F, Cl, Br, I or SO_3H;

R_{12} is H, OH, OR or OCOR; and

R is an alkyl or substituted alkyl group.

It is noted that the compound of formula II wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, and R_9 and R_{10} are methyl is hypericin itself. It is also noted that the compound of formula II wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_9 is methyl and R_{10} is hydroxymethyl is pseudohypericin.

In the present invention, "alkyl" represents a C_1-C_{30} linear or branched saturated or unsaturated hydrocarbyl group. Preferably, "alkyl" is a C_1-C_6 linear or branched saturated or unsaturated hydrocarbyl group.

The alkyl group of R can be optionally substituted with one to three substituents independently selected from hydroxy, alkoxy, acyloxy, carboxy, akoxycarbonyl, amino, alkylamino, dialkylamino, nitro or phenyl

group or fluorine, chlorine, bromine or iodine atom.

Further preferred are compounds of formula II, wherein

R_1 is H, OH, OR or OCOR;

R_2 is H or R;

R_3 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_4 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_5 is H or R;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H or R;

R_9 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{10} is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{11} is H or R;

R_{12} is H, OH, OR or OCOR; and

R is an optionally substituted C_1-C_6 alkyl group, as well as methods of using these compounds to treat health disorders treatable with T-calcium channel blockers.

It is also preferred that R is an optionally substituted methyl or ethyl group.

The hypericin derivatives of the present invention with alkyl or substituted alkyl group(s), i.e. compounds of formula II with alkyl or substituted alkyl group(s) at R_2, R_3, R_4, R_5, R_8, R_9, R_{10}, and/or R_{11} position, can be synthesized from appropriately substituted emodin anthones by dimerization (see Y. Mazur et al, CA 2,029,993; H. Falk et al, *Monatsh. Chem.*, vol. 126, pp. 993-1000, 1995; H. Falk and T.N.H. Tran, *Monatsh. Chem.*, vol. 127, pp. 717-723, 1996; R. Altmann et al, *Monatsh. Chem.*, vol. 129, pp. 235-244, 1998; G.A. Kraus and W. Zhang, *Bioorg. Med. Chem.*, vol. 5, pp. 2633-2636, 1995). For example, a hypericin derivative, wherein R_1, R_3, R_4, R_6, R_7, and R_{12} are OH, R_2, R_5, R_8, and R_{11} are H, and R_9 and R_{10} are $C_{19}H_{39}$, was synthesized from anthone of formula I by dimerization in the presence of pyridine N-oxide, piperidine, and ferrous

sulfate in pyridine.

O-Substituted hypericin derivatives or hypericin analogs can be synthesized by direct etherification and esterification of the phenolic hydroxyl group of hypericin and/or hypericin analogs (see H. Falk and T.N.H. Tran, *Monatsh. Chem.*, vol. 127, pp. 717-723, 1996; G.A. Kraus and W. Zhang, *Bioorg. Med. Chem.*, vol. 5, pp. 2633-2636, 1995). For example, a hypericin derivative, wherein R_1, R_3, R_4, R_6, R_7, and R_{12} are OMe, R_2, R_5, R_8, and R_{11} are H, and R_9 and R_{10} are methyl, was prepared by methylation of hypericin with dimethyl sulfate under basic condition.

Hypericin derivatives having halogen or sulfonate substitution, i.e. compounds of formula II wherein R_2, R_5, R_8, and/or R_{11} are halogen or SO3H, or halogenated or sulfonated hypericin analogs can be synthesized by direct halogenation or sulfonation of hypericin or hypericin analog (H. Falk and W. Schmitzberger, *Monatsh. Chem.*, vol. 124, pp. 77-81, 1993; H. Falk et al, *Monatsh. Chem.*, vol. 129, pp. 309-318, 1998). For example, a hypericin derivative, wherein R_2, R_5, R_8, and/or R_{11}=Br, was prepared by bromination of hypericin in pyridine.

Other hypericin derivatives of formula II can be prepared by derivatization of hypericin, pseudohypericin or the hypericin derivatives described above using processes known in the art. For instance, one or more of the hydroxy groups in hypericin, pseudohypericin or the hypericin derivatives described above can be derivatized by converting the hydroxy group(s) to a protected hydroxy group(s) according to the processes described in T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc., New York, 1991, the disclosure of which is incorporated by reference. Similarly, hypericin derivatives can be prepared by converting hypericin or pseudohypericin into a prodrug of hypericin or pseudohypericin using processes known in the art, e.g. the processes described in H. Bundgaard, *Design of Prodrugs*, Elsevier Science Publishers, Amsterdam, 1985, the disclosure of which is

incorporated by reference. The present invention includes prodrug forms of the agents disclosed above and methods of using the prodrug forms to treat health disorders related to T-type calcium channels or treatable with T-calcium channel blockers. For instance, hypericin prodrugs, pseudohypericin prodrugs and methods of using hypericin prodrugs or pseudohypericin prodrugs to treat health disorders related to T-type calcium channels or treatable with T-calcium channel blockers are also contemplated in the present invention.

The present invention also provides pharmaceutical compositions comprising *Hypericum, Hypericum* extract, or a compound found in *Hypericum*, i.e. *Hypericum* constituent, including hypericin, pseudohypericin, hyperforin, ashyperforin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, hypericin derivatives or hypericin analogs, admixed with a pharmaceutically acceptable carrier. Also within the scope of the present invention is a pharmaceutical composition comprising at least two *Hypericum* constituents, one of which is preferably hypericin, with or without a pharmaceutically acceptable carrier. It is further preferred that the pharmaceutical composition comprises hypericin and pseudohypericin. It is also preferred that the pharmaceutical composition comprises hypericin and hyperforin. A further aspect of the present invention is a pharmaceutical composition comprising a *Hypericum* constituent and a hypericin derivative or hypericin analog.

Numerous standardized extracts are available yielding from 0.4 to 2.7 mg of hypericin per daily dose. These are prepared in a variety of ways according to the various manufacturers. The extracts are normally standardized by containing 0.24-0.32% total hypericin.

The primary compounds, hypericin and pseudohypericin (naphthodianthrones), are naturally occurring pigments and characteristic markers for *Hypericum* plant and can be extracted in methanol or ethanol. Hypericin and pseudohypericin can also be obtained synthetically by

processes known in the art. Synthetic hypericin is also available commercially. Similarly, *Hypericum* extract constituents, such as hypericin, pseudohypericin, flavonoids, phloroglucinols and xanthones, can be obtained by synthetic processes known in the art or by high pressure liquid chromatography of *Hypericum* extracts, followed by work-up procedures known to one skilled in the art.

The present invention also provides methods of using *Hypericum*; *Hypericum* extracts; *Hypericum* constituents, e.g. hypericin, pseudohypericin, flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, phloroglucinols, such as hyperforin and ashyperforin, the essential oil from *Hypericum perforatum*, and xanthones; hypericin derivatives; hypericin analogs; or the pharmaceutical compositions disclosed above for treating health disorders related to T-type calcium channels or treatable with T-calcium channel blocksers. In these methods, *Hypericum*, *Hypericum* extract, hypericin, pseudohypericin, flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, phloroglucinols, such as hyperforin and ashyperforin, the essential oil from *Hypericum perforatum*, xanthones, hypericin derivatives, hypericin analogs, or the pharmaceutical compositions disclosed above may be administered to an animal, e.g. a mammal such as a human, in need of such a treatment by a parenteral, opthalmological, topical, oral or rectal route or by inhalation. Examples of parenteral route are intravenous, subcutaneous and intramuscular routes.

Hypericum extract, hypericin, pseudohypericin, flavonoids, such as quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone and hyperin, phloroglucinols, such as hyperforin and ashyperforin, the essential oil from *Hypericum perforatum*, and xanthones, hypericin derivatives, or hypericin analogs may be formulated for parenteral, ophthalmological, topical, oral or rectal administration by compounding these active agents with a conventional vehicle, excipient, binder, preservative, stabilizer, dye, flavoring agent, or the like, as called for by

accepted pharmaceutical practice.

The doses of the active agents used in the methods of the present invention are described herein. Daily doses are in the range of 0.05 to 500 mg per kg of body weight, prefereably 0.5 to 50 mg per kg of body weight, for *Hypericum* extract, an extract of a species of the genus *Hypericum* other than *Hypericum perforatum*, or an extract of a plant containing hypericin. Daily doses are in the range of 0.0001 to 10 mg per kg of body weight, preferably 0.0015 to 0.15 mg per kg of body weight, for hypericin and 0.001 to 5 mg per kg of body weight for a hypericin derivative, such as pseudohypericin, or hypericin analog. For *Hypericum* extract constituents or *Hypericum* constituents, other than hypericin and pseudohypericin, the daily doses are in the range of 0.01 to 100 mg per kg of body weight, preferably of 0.05 to 50 mg per kg of body weight.

Hypericum perforatum, fresh or dried, can be used in the methods of treating health disorders related to T-type calcium channels or treatable with T-calcium channel blockers. In the present invention, "fresh *Hypericum perforatum*" includes the entire plant of *Hypericum perforatum* or a portion of *Hypericum perforatum* plant in a fresh state. In the present invention, "dried *Hypericum perforatum*" includes the entire plant of *Hypericum perforatum* or a portion of *Hypericum perforatum* plant in a dry state, as well as powder resulting from grinding a dried *Hypericum perforatum* plant or a dried portion of a *Hypericum perforatum* plant. The methods of treatment of the present invention can be performed by administering fresh *Hypericum perforatum* or dried *Hypericum perforatum*. For fresh *Hypericum perforatum*, the daily doses are in the range of 1 to 5000 mg per kg of body weight. For dried *Hypericum perforatum*, the daily doses range from 0.5 to 2000 mg per kg of body weight.

The actual dose of the active agent used in the method of the present invention to treat a particular subject can be selected from the "daily doses" disclosed above depending on the T-calcium channel activity of the active agent, i.e. *Hypericum perforatum*, *Hypericum* extracts,

Hypericum constituents, hypericin derivatives or hypericine analogs, used in the treatment, the age, race, sex, species and health condition of the subject to be treated and the type and severity of the health disorder to be treated. If used for acute treatments, the above active agents can be administered at the daily doses disclosed above for no more than one day. If needed, the above active agents can be administered to the subject being treated at the above daily doses repetitively day after day.

The extract or hypericin can also be combined with drugs or any other natural substances known to be effective for treating the condition in question.

The following examples illustrate, but are not intended to limit, the present invention.

Example 1.

Effect of hypericin on L-type calcium current activity in cultured neuroblastoma cells.

Mouse neuroblastoma cells (N1E115) were cultured in Dulbecco's modified Eagle's medium (GIBCO) containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 in air. The medium was changed every 3-4 days. After mechanical agitation, 3 x 104 cells were replanted in 35 mm tissue culture dishes containing 4 ml of bath solution. After cell attachment, the dish was mounted on the stage of an inverted phase-contrast microscope (Nikon) for Ca^{2+} channel current recording. These cells expressed predominately T channel currents. In experiments where L channels were specifically sought, the cells were grown and maintained at confluence for 3-4 weeks under the same culture conditions with the addition of 2% (vol/vol) dimethyl sulfoxide. Three to five days before use, the cells were replanted with the same medium. These cells expressed predominately L channel currents. A small number of these cells also expressed T channel currents. Hence, cells were selected so that at a holding potential of –40 mV, the T channel component was very small and the inward current measured was conducted predominantly by L channels.

The whole-cell version of the patch-clamp technique was used. The pipettes had resistance of 2-15 M. Membrane current recordings were made with an Axopatch-1C (Axon Instruments) patch-clamp amplifier. All signals were filtered at 1 kHz and stored in the computer. Since the peak currents measured with 20 mM Ba^{2+} as the charge carrier were usually small (about 200 pA), the series resistance compensation was not usually employed. If the capacitive transient overlapped with the onset of the inward current, or if the spatial voltage control was inadequate (i.e., NIE-115 cells with long neural outgrowths), the experimental data were rejected. Unless otherwise specified the current-voltage plots were constructed by using the peak values (corrected for leakage) from the original records for both T and L channel currents. The holding membrane potential was fixed at –80 mV when the T channels were under investigation or at –40 mV when the L channels were studied. Ba^{2+} currents through Ca^{2+} channels were elicited by 200 msec depolarization at intervals of 5 sec. For every single-cell recording, stable readings were first obtained for 5 min; the drug was then added to the bath solution. Experiments were performed at room temperature(21-22°C) to prolong cell survival and channel recording time. The bath solution contained 110 mM Tris, 5 mM KCL, 5 mM CsCL, 20 mM Hepes, 30 mM glucose, 20 mM $BaCL_2$, and 0.5 M tetrodotoxin. The pipette (internal) solution contained 70 mM Cs_2–aspartame, EGTA 10, 2 mM ATP-Na_2, 5 mM K-pyruvate, 5 mM K-succinate, 5 mM Phosphocreatine-Na_2, 15 units/ml Creatine kinase, Hepes and 5 mM glucose. The osmolarity of all solutions was adjusted to 310-320 mOsm and pH to 7.4 using HCL or CsOH as required.

Figure 1 shows that hypericin does not significantly affect L-type calcium current. Figure 2 shows that Nifedipine, a known L-type calcium channel blocker significantly inhibits L-type calcium currents.

Example 2.

Effect of Hypericin on T-type Calcium Currents in N1E-115 cells.

The method is described as above (Example 1). Figure 3 shows that hypericin inhibited T-type calcium currents in a dose-dependent manner.

Figure 4 shows that the solvent controls did not affect the T-type calcium currents.

Example 3.

Effect of St. John's Wort extract (*Hypericum* extract) on T-type calcium current in N1E-115 Cells.

The method is described as above (Example 1). *Hypericum* extract is standardized with about 0.3% of hypericin. As shown in Figure 5, at, 50 ug/ml, *Hypericum* extract containing about 0.15 ug/ml of hypericin significantly inhibited T-type calcium current by more than 60%. However, 0.15 ug/ml of pure hypericin, as shown in Figure 3 produces less than 10% of inhibition on T-type calcium current. This results suggested that the chemicals other than hypericin in *Hypericum* extract cause a synergistic effect to hypericin on T-type calcium channel activity.

Example 4

Effect of Hypericin on L-type calcium current in VSMC

The method is similar to the method described above for Example 1, except that vascular smooth muscle cells (VSMC) were used. As shown in Fig. 6, hypericin did not affect the L-type calcium current in vascular smooth muscle cells.

Example 5

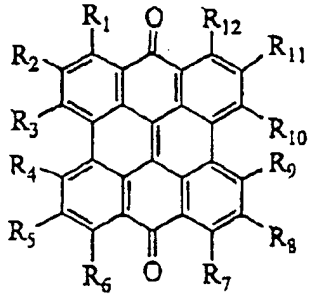
Effect of Hypericin on L-type calcium current in Ventricular Cells

The method is similar to the method described above for Example 1, except that ventricular cells from baby rats were used. As shown in Fig. 7, hypericin did not affect the L-type calcium current in ventricular cells.

What is claimed is:

1. A method of treating a health disorder treatable with a T-type calcium channel blocker in an animal in need of such a treatment, comprising administering an effective amount of an active agent to said animal, wherein said active agent is *Hypericum perforatum*, a *Hypericum* extract, an extract of a species of the *Hypericum* genus other than *Hypericum perforatum*, a *Hypericum* constituent, a hypericin derivative or a hypericin analog, with the proviso that when the active agent is *Hypericum perforatum* or *Hypericum* extract, said health disorder is not depression or migraine headache.

2. The method of claim 1, wherein said hypericin derivative is a compound of formula II,



II

wherein

R_1 is H, OH, OR or OCOR;

R_2 is H, R, F, Cl, Br, I or SO_3H;

R_3 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_4 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_5 is H, R, F, Cl, Br, I or SO_3H;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H, R, F, Cl, Br, I or SO_3H;

R_9 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{10} is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{11} is H, R, F, Cl, Br, I or SO_3H;

R_{12} is H, OH, OR or OCOR; and

R is an optionally substituted C_1-C_{30} alkyl group;

with the proviso that the following compounds are excluded

(A) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, and R_9 and R_{10} are methyl;

(B) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, and R_3 and R_4 are methyl;

(C) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_9 is methyl, and R_{10} is CH_2OH;

(D) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_9 is CH_2OH and R_{10} is methyl;

(E) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_3 is methyl, and R_4 is CH_2OH; and

(F) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_3 is CH_2OH and R_4 is methyl.

3. The method of claim 1, wherein the health disorder treatable with T-type calcium channel blockers is depression, chronic heart failure, congestive heart failure, ischaemc condition, arrhythmia, angina pectoris, hypertension, hypoinsulinemia, hyperinsulinemia, diabete mellitus, hyperaldosteronemia, epilepsy, migraine headache, brain aging, a neurodegenerative disease or preterm labor.

4. The method of claim 1, wherein said *Hypericum* constituent is hypericin, pseudohypericin, hyperforin, ashyperforin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, amentoflavone or hyperin.

5. The method of claim 2, wherein R is a C_1-C_{30} alkyl group, optionally substituted with one to three substituents independently selected from hydroxy, alkoxy, acyloxy, carboxy, akoxycarbonyl, amino,

alkylamino, dialkylamino, nitro or phenyl group or fluorine, chlorine, bromine or iodine atom.

6. The method of claim 5, wherein

R_1 is H, OH, OR or OCOR;

R_2 is H or R;

R_3 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_4 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_5 is H or R;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H or R;

R_9 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{10} is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{11} is H or R;

R_{12} is H, OH, OR or OCOR; and

R is an optionally substituted C_1-C_6 alkyl group.

7. The method of claim 6, wherein R is an optionally substituted methyl or ethyl group.

8. The method of claim 1, wherein said animal is a human.

9. The method of claim 1, wherein said active agent is a *Hypericum* extract.

10. The method of claim 9, wherein said effective amount is about 0.05 mg to 500 mg per kg body weight of said animal.

11. The method of claim 1, wherein said active agent is hypericin.

12. The method of claim 11, wherein said effective amount is about 0.0015 mg to 15 mg per kg body weight of said animal.

13. The method of claim 1, further comprising administering to said animal an additional active agent as described in claim 1.

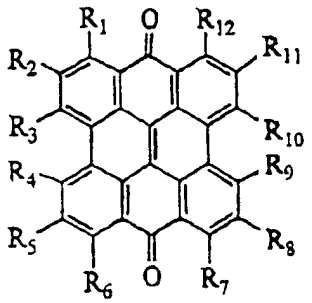
14. The method of claim 13, wherein one of the active agents administered is hypericin.

15. The method of claim 14, wherein another of the active agents administered is pseudohypericin.

16. The method of claim 14, wherein another of the active agents administered is hyperforin.

17. The method of claim 15, further comprising administering hyperforin to said animal.

18. A compound of formula II,



II

wherein

R_1 is H, OH, OR or OCOR;

R_2 is H, R, F, Cl, Br, I or SO_3H;

R_3 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_4 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_5 is H, R, F, Cl, Br, I or SO_3H;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H, R, F, Cl, Br, I or SO_3H;

R_9 is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{10} is H, R, OH, OR, OCOR, CH_2OH, CH_2OR, CH_2OCOR, COOH or COOR;

R_{11} is H, R, F, Cl, Br, I or SO_3H;

R_{12} is H, OH, OR or OCOR; and

R is an optionally substituted C_1-C_{30} alkyl group;

with the proviso that the following compounds are excluded

(A) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, and R_9 and R_{10} are methyl;

(B) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, and R_3 and R_4 are methyl;

(C) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_9 is methyl, and R_{10} is CH_2OH;

(D) a compound of formula II, wherein R_1, R_3, R_4, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_9 is CH_2OH and R_{10} is methyl;

(E) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_3 is methyl, and R_4 is CH_2OH;

(F) a compound of formula II, wherein R_1, R_9, R_{10}, R_6, R_7 and R_{12} are OH, R_2, R_5, R_8 and R_{11} are H, R_3 is CH_2OH and R_4 is methyl.

19. The compound of claim 18, wherein R is a C_1-C_{30} alkyl group, optionally substituted with one to three substituents independently selected from hydroxy, alkoxy, acyloxy, carboxy, akoxycarbonyl, amino, alkylamino, dialkylamino, nitro or phenyl group or fluorine, chlorine, bromine or iodine atom.

20. The compound of claim 18, wherein

R_1 is H, OH, OR or OCOR;

R_2 is H or R;

R_3 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_4 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_5 is H or R;

R_6 is H, OH, OR or OCOR;

R_7 is H, OH, OR or OCOR;

R_8 is H or R;

R_9 is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{10} is H, OH, OR, OCOR, CH_2OH, CH_2OR or CH_2OCOR;

R_{11} is H or R;

R_{12} is H, OH, OR or OCOR; and

R is an optionally substituted C_1-C_6 alkyl group.

21. The compound of claim 20, wherein R is an optionally substituted methyl or ethyl group.

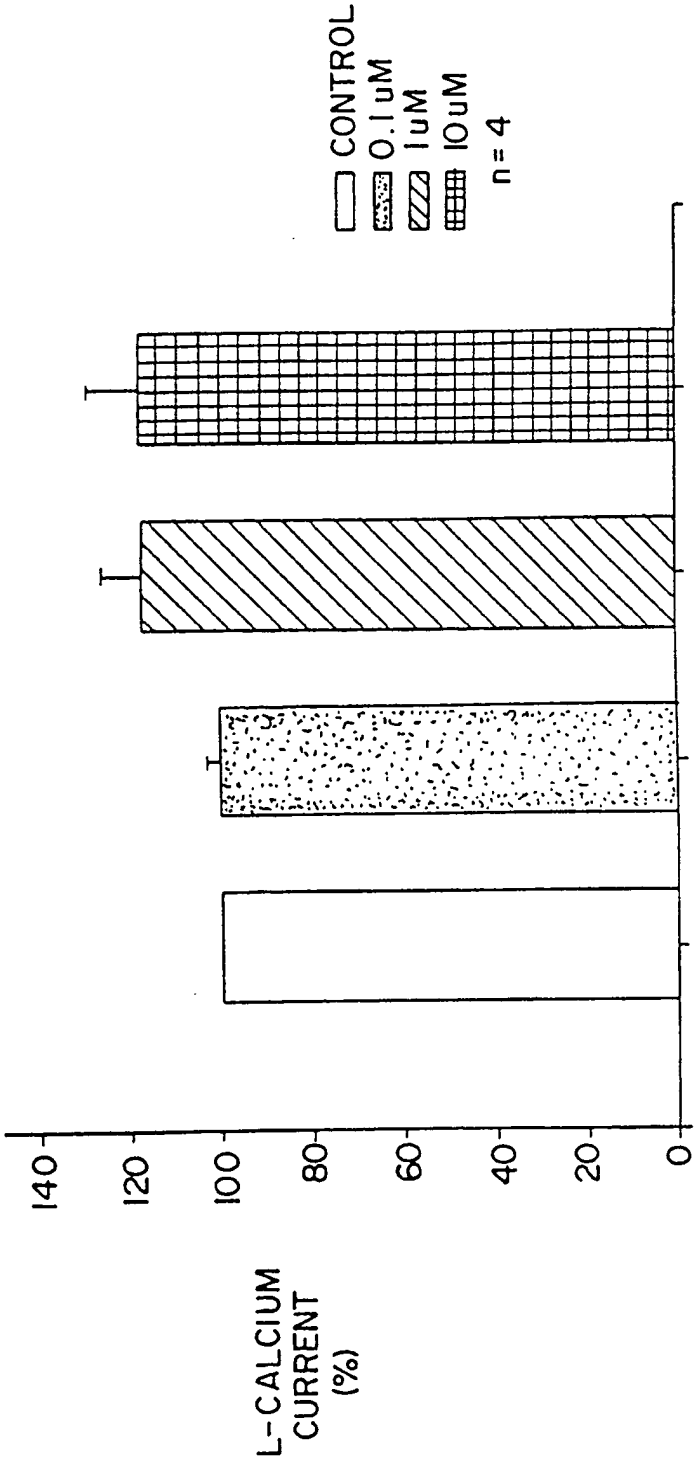

CONTROL
0.1uM
1uM
10uM
n=4
140
120
100
80
60
40
20
0
L-CALCIUM CURRENT (%)

Fig. 1

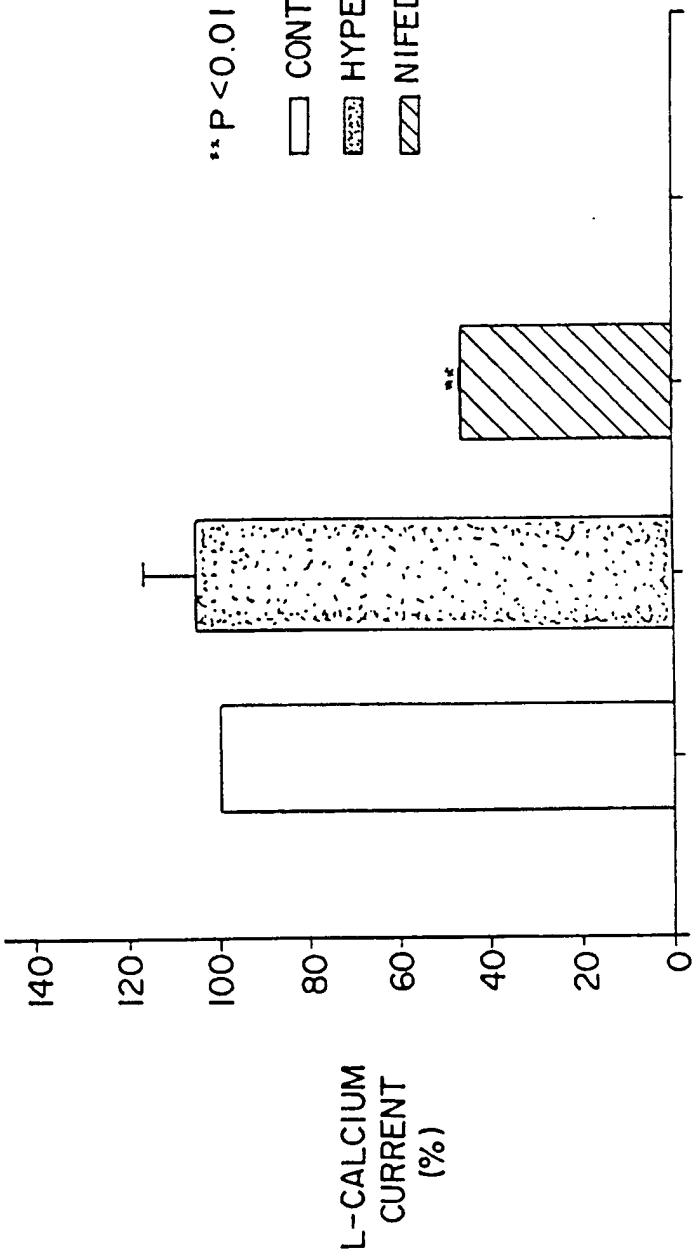

** P<0.01
CONTROL
HYPERICIN 10uM
NIFEDIPINE 1uM
L-CALCIUM CURRENT (%)
140
120
100
80
60
40
20
0

Fig. 2

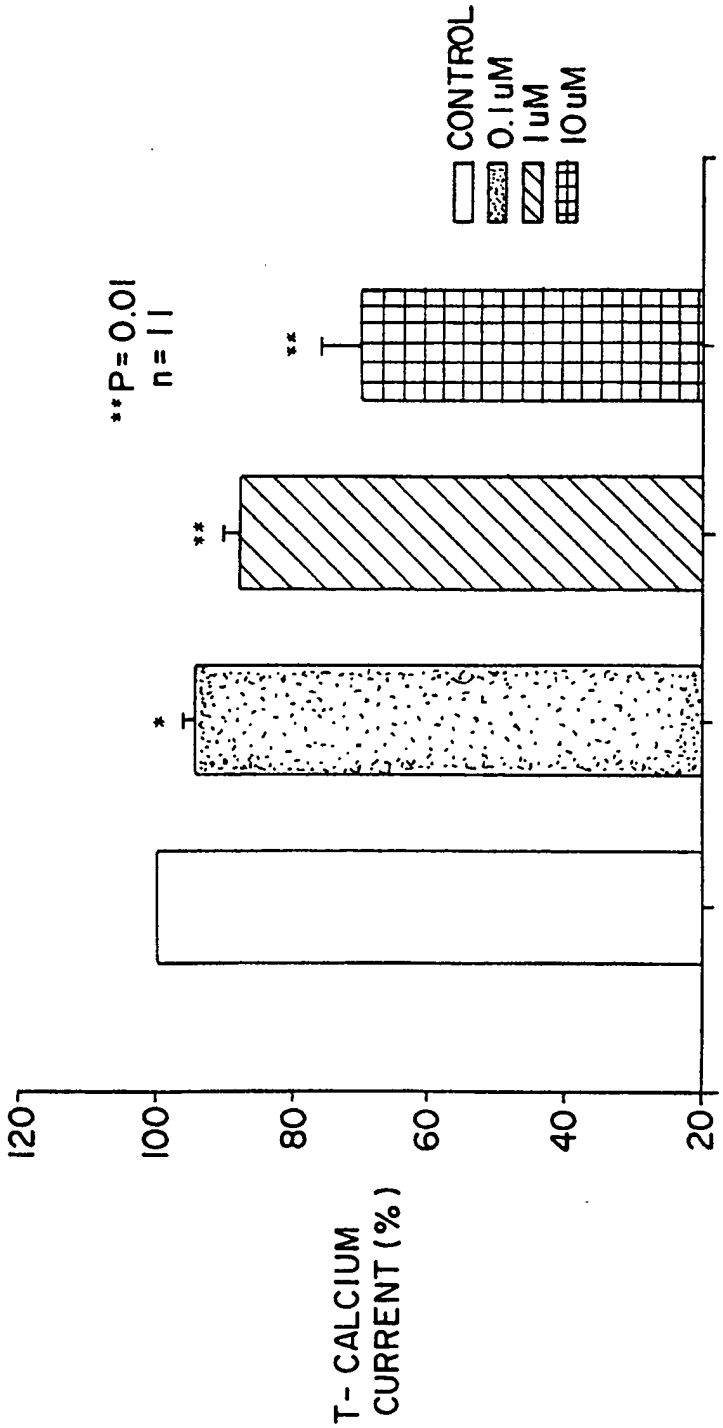

CONTROL
0.1uM
1uM
10uM
**P=0.01
n=11
*
**
**
120
100
80
60
40
20
T- CALCIUM
CURRENT (%)

Fig. 3

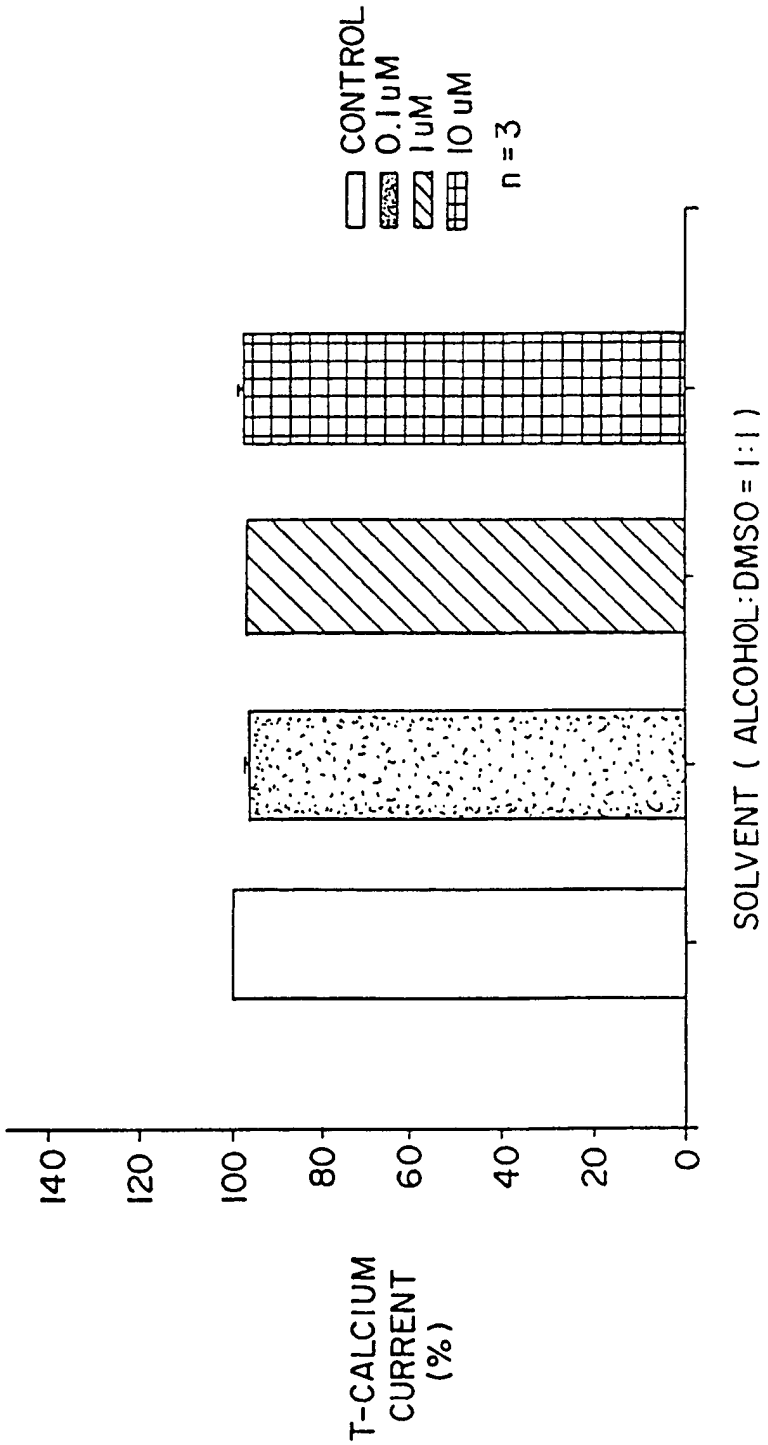

CONTROL
0.1uM
1uM
10uM
n = 3
T-CALCIUM CURRENT (%)
140
120
100
80
60
40
20
0
SOLVENT (ALCOHOL:DMSO = 1:1)

Fig. 4

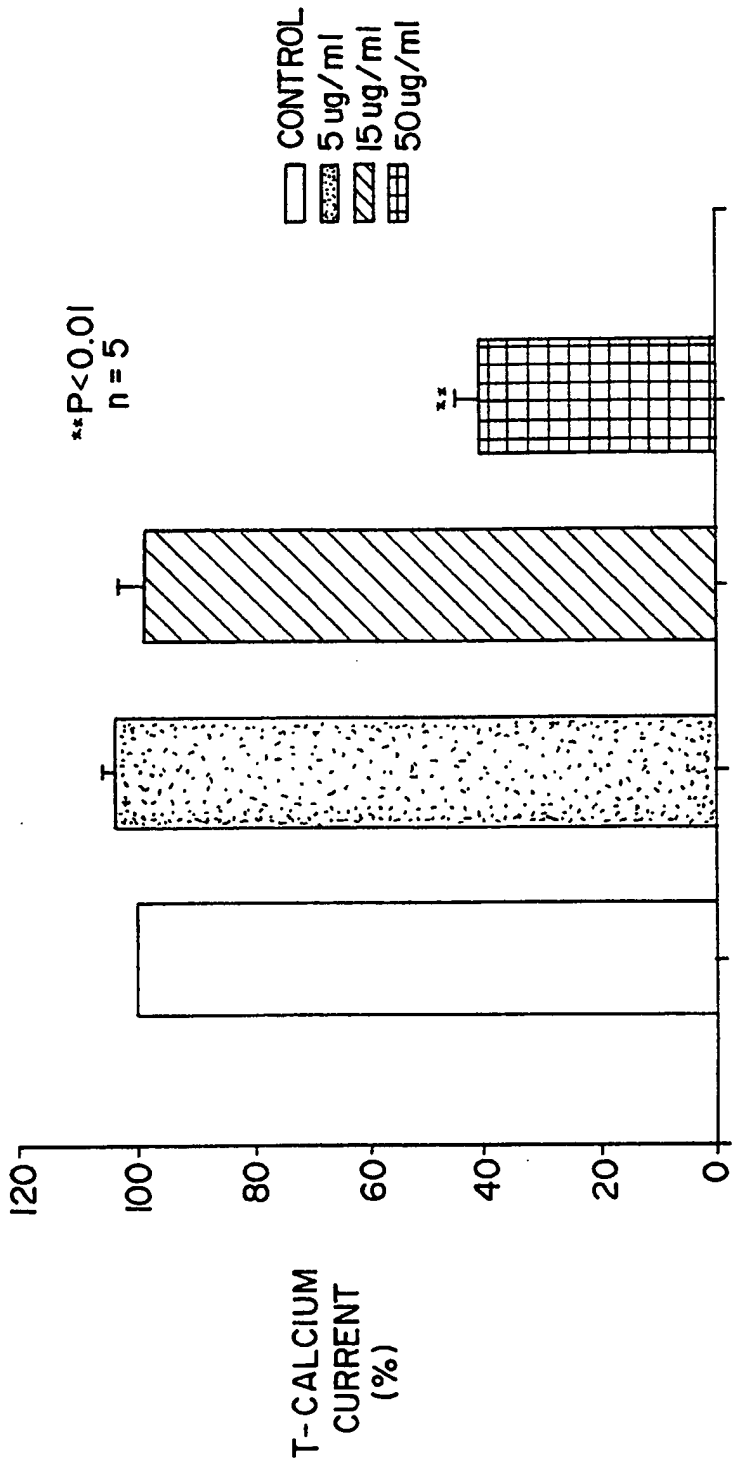

CONTROL
5 ug/ml
15 ug/ml
50 ug/ml
**P<0.01
n=5
**
T
T
T
T-CALCIUM CURRENT (%)
120
100
80
60
40
20
0

Fig. 5

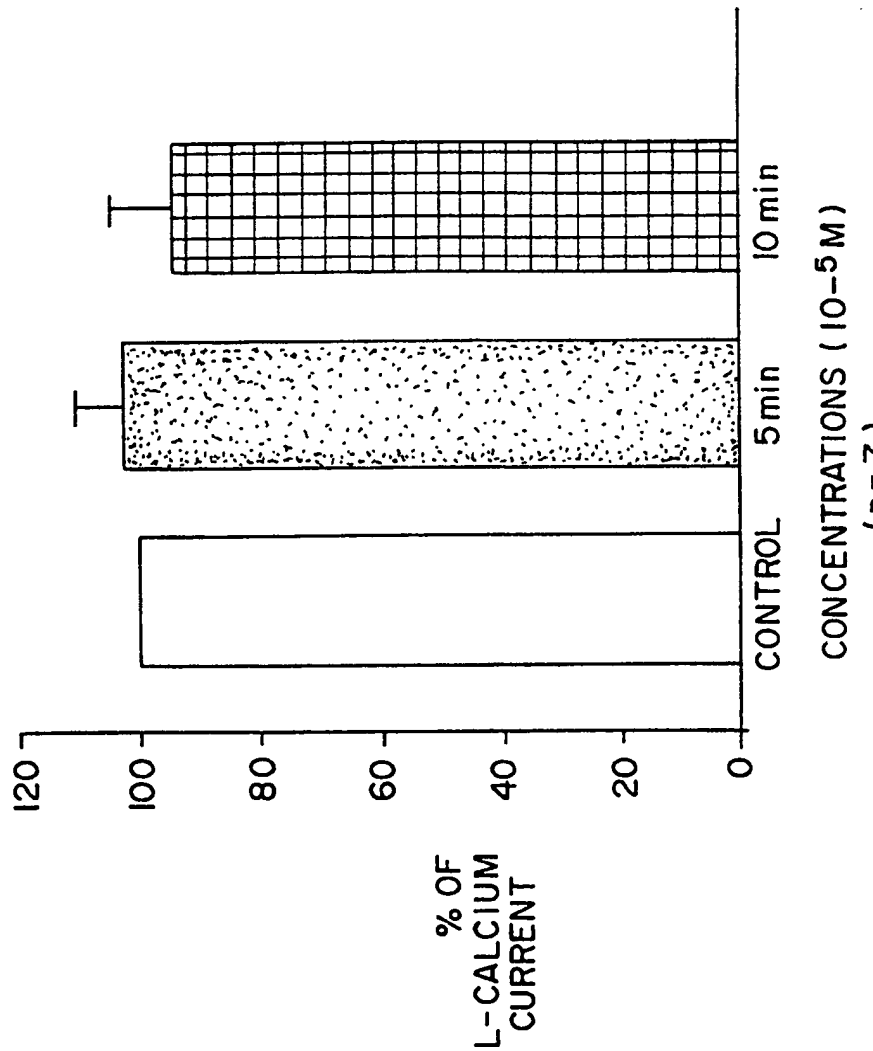

% OF L-CALCIUM CURRENT
120
100
80
60
40
20
0
CONTROL
5min
10 min
CONCENTRATIONS (10-5M)
(n=7)

Fig. 6

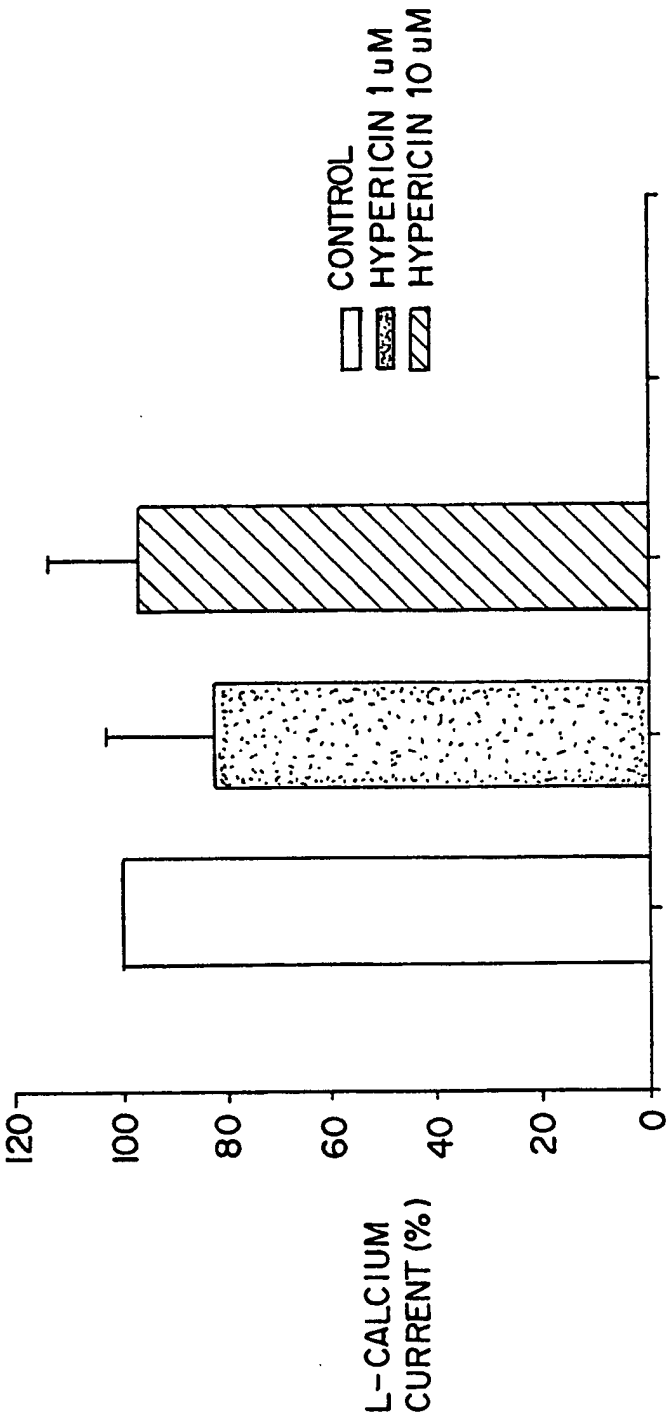

CONTROL
HYPERICIN 1 uM
HYPERICIN 10 uM
L-CALCIUM CURRENT (%)
120
100
80
60
40
20
0

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/14132

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 65/00, 35/00, 29/00; C07C 17/00, 19/08, 22/00
US CL :424/195.1; 514/678, 688, 743, 765; 562/74; 570/101, 123, 127, 182

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1; 514/678, 688, 743, 765; 562/74; 570/101, 123, 127, 182

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, MEDLINE, BIOSIS, EMBASE, REGISTRY, SCISEARCH, CAPLUS
search terms: depression, ischemia, epilepsy, Hypericum, hyperforin, hypericin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 5,120,412 A (MAZUR et al) 09 June 1992, see columns 3 and 4.	1-9, 1-14, 18-21 --- 15-17
X - Y	US 5,433,957 A (KIKUTA et al) 18 July 1995, see columns 3 to 4.	1, 3, 8, 9 --- 2, 4-7, 10-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 AUGUST 1999	Date of mailing of the international search report 03SEP1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUSAN HANLEY Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/14132

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOAMESI et al. Anticonvulsant Effect of Aqueous and Ethanol Extracts of Mareya spicata Against Pentylenetetrazole, Lidocaine, and Aminophylline Induced Convulsions in Mice. Planta Medica. 1991, Vol. 57, Suppl. 1, page A55, see second paragraph.	1, 4